User Guide

Catalog Nos.
NA0300
NA0300S
NA0310

GenElute™ HP Plasmid Maxiprep Kit

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Ordering Information

Cat. No.	Product Description	Pkg Size
NA0150	GenElute HP Plasmid Miniprep Kit	70 preps
NA0200S	GenElute HP Plasmid Midiprep Kit	4 preps
NA0200	GenElute HP Plasmid Midiprep Kit	25 preps
NA0300S	GenElute HP Plasmid Maxiprep Kit	4 preps
NA0300	GenElute HP Plasmid Maxiprep Kit	10 preps
NA0310	GenElute HP Plasmid Maxiprep Kit	25 preps
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GenElute™ High Performance (HP) Plasmid Maxiprep Kit

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Product Description

Sigma's GenElute HP Plasmid Maxiprep Kits offer a simple, rapid, and cost-effective method for isolating plasmid DNA from recombinant *E. coli* cultures. The kits feature a filter syringe for the rapid clearing of lysate and a silica binding column designed for either a vacuum or a spin format. Up to 1.2 mg of plasmid DNA can be isolated from a 150 mL overnight culture grown in Luria Broth (LB) medium. Note that the actual yield depends on the strain, the plasmid, and the culture medium used.

An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis procedure followed by adsorption of the DNA onto a silica membrane in the presence of high salts.[1,2] Contaminants are removed by two wash steps. Finally, the bound DNA is eluted in Elution Solution (Tris-HCl) or water.

The recovered plasmid DNA is predominately in its supercoiled form. Genomic DNA or RNA are below detectable levels by ethidium bromide stained agarose gel electrophoresis. The DNA is ready for immediate use in downstream applications such as restriction digestion, ligation, sequencing, PCR, transformation, and transfection.

Reagents Provided	Cat. No.	NA0300S 4 Preps	NA0300 10 Preps	NA0310 25 Preps
Column Preparation Solution	**C2112**	225 mL	225 mL	2 × 225 mL
RNase A Solution	**R6148**	1.5 mL	1.5 mL	2.5 mL
Resuspension Solution	**R1149**	150 mL	150 mL	375 mL
Lysis Solution	**L1912**	150 mL	150 mL	375 mL
Neutralization Solution	**N1285**	150 mL	150 mL	375 mL
Binding Solution	**B4683**	110 mL	110 mL	280 mL
Wash Solution 1	**W0263**	150 mL	150 mL	375 mL
Wash Solution 2	**W4639**	30 mL	30 mL	75 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	**E7777**	45 mL	45 mL	115 mL
GenElute HP Maxiprep Filter Syringe	**G9042**	4	10	25
GenElute HP Maxiprep Binding Column	**G4917**	4	10	25
Collection Tubes, 50 mL conical	**C4353**	8	20	50

Equipment and Reagents Required But Not Provided

- Ethanol (95–100%), Catalog No. **E7148**, **E7023**, or **459836**
- Centrifuge capable of 5000 × *g*
- Centrifuge with a swinging bucket rotor capable of 3000 × *g*
- Vacuum Manifold, Catalog No. **VM20**

Precautions and Disclaimer

The GenElute HP Plasmid Maxiprep Kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Storage and Stability

Store the kit at room temperature. Once the RNase A Solution is added to the Resuspension Solution, store at 2–8 °C. The Neutralization Solution can also be stored at 2–8 °C, since it is recommended to use this solution chilled in the protocol.

Preparation Instructions

1. **Prepare a starter culture**

   Pick a single colony from a freshly streaked plate and inoculate a starter culture of 3 to 5 ml LB medium. Use the appropriate antibiotic and incubate at 37 °C for approximately 8 hours while shaking at 250–300 rpm. Dilute the starter culture 1:500 to 1:1000 in the appropriate volume of LB medium and incubate at 37 °C for 12 to 16 hours while shaking at 250–300 rpm.

2. **Choosing the correct culture volume**

   Use of **150 ml** of culture generally results in good plasmid yields. However, the optimal volume of culture to use depends upon the strain, the plasmid, and the density of the culture since the number of bacterial cells can vary greatly between cultures.

   Too few cells (low cell mass) will result in low DNA yields and may cause a very fine flocculent after neutralization that could cause clogging during filtration. Conversely, with too many cells (high cell mass) the bacteria may not lyse efficiently and cause poor release of the plasmid DNA or the potential to trap lysate volume in the cell debris during filtration resulting in a lower yield. By following the cell mass calculation, you will ensure maximum plasmid recovery from the overnight culture.

   For best results, we recommend using a volume of culture based on cell mass. A total cell mass of **750 is typically optimal**. The optimal volume of culture to use can be determined by measuring the absorbance of the overnight culture at 600 nm (A_{600}) and using the formula below:

   $$\text{Volume}_{\text{optimal}} = \frac{750}{A_{600}}$$

3. **Thoroughly Mix Reagents**

   Examine the reagents for precipitation. If any reagent forms a precipitate upon storage, warm at 55–65 °C until the precipitate dissolves. Allow the reagent to cool to room temperature before use.

4. **Prepare Resuspension Solution + RNase A**

   Spin the tube of RNase A Solution briefly. Add 750 µL (4 and 10 prep kit) or 1.9 mL (25 prep kit) of the RNase A Solution to the Resuspension Solution prior to initial use. Store at 4 °C.

5. **Dilute Wash Solution 2**

   Dilute Wash Solution 2 with 120 mL (4 and 10 prep kit) or 300 mL (25 prep kit) of 95–100% ethanol prior to initial use. After each use, tightly cap diluted Wash Solution 2 to prevent evaporation of the ethanol.

Procedure

All steps are carried out at room temperature. When using a vacuum, make certain the vacuum level is equal to or greater than 500 mbar (refer to Appendix 2 for unit conversions).

Convenient stopping points

Step 1:

The wet bacterial pellet can be frozen at –70 ºC for one month without any detrimental effects to the quality or yield of the plasmid DNA.

Step 7 and step 8:

Do not prepare Binding Column in Step 7 (a or b). Instead perform Steps 1-6 and 8 (a or b). **Do not use Binding Column in Step 8!** Instead, collect the filtered lysate with clean appropriate size container, such as polypropylene tube. Now cleared lysate containing Binding Solution can be stored overnight at 2-8 ºC without any detrimental effects to the quality or yield of the plasmid DNA.

When you are ready to continue the plasmid purifications, prepare the Binding Column with Column Preparation Solution (C2112) as described in Step 7 (a or b), then load the cleared lysate containing Binding Solution to the column and follow the procedure to finish the DNA preparation.

1. Harvest Cells

Important Reminder: *The optimal volume of culture can be calculated based on cell mass. Refer to Preparation Instructions.*

Pellet **150 mL of an overnight culture** by centrifugation at **5000 X *g* for 10** minutes and discard the supernatant.

2. Resuspend Cells

Important Reminder: *Verify that RNase A Solution was added to the Resuspension Solution.*

Add **12 ml of Resuspension/RNase A Solution** to the bacterial pellet and completely resuspend by pipetting up and down, or vortexing.

Incomplete resuspension can result in poor recovery of plasmid DNA.

3. Lyse Cells

Lyse the resuspended cells by adding **12 ml of Lysis Solution**. Immediately mix the contents by gently inverting 6 to 8 times. Let the mixture sit for 3 to 5 minutes until it becomes clear and viscous.

Do not shake or vortex. *Harsh mixing will shear genomic DNA and may contaminate the final recovered plasmid DNA.*

Do not allow lysis to proceed longer than 5 minutes. *Prolonged alkaline lysis may permanently denature the supercoiled plasmid DNA and may render it unsuitable for use in downstream applications.*

4. Prepare Filter Syringe

Prepare a filter syringe by removing the plunger and placing the barrel in a rack so that the syringe barrel is upright.

5. Neutralize

Important Reminder: *Confirm that Neutralization Solution is chilled to 2-8 ° C.*

Neutralize the lysed cells from Step 3 by adding **12 ml of chilled Neutralization Solution** to the mixture and gently invert 4 to 6 times. A white aggregate (cell debris, proteins, lipids, SDS, and chromosomal DNA) will form.

6. Add Binding Solution

Add **9 mL of Binding Solution** and invert 1 to 2 times. Immediately pour into the barrel of the filter syringe. The cell lysate will not pass through the filters until the plunger is inserted into the syringe. Allow the lysate to **sit for 5 minutes**. The white aggregate should float to the top. During incubation, proceed to the next step using either the vacuum (7a) or the spin (7b) format.

Vacuum Format

7a. Prepare Binding Column

Place a GenElute HP Maxiprep Binding Column onto the vacuum manifold and apply vacuum. Add **12 ml of Column Preparation Solution** to the column and allow it to pass through. *For convenience, this step can be performed during one of the previous incubation steps*.

8a. Filter lysate and bind DNA to column

Hold the filter syringe barrel over the binding column and gently apply pressure to the plunger to expel the cleared lysate into the column.

Be careful not to overfill. Allow the lysate to pass through the column. *Some of the lysate may remain in the flocculent material. It is not necessary to force this residual lysate through the filter syringe*.

9a. Apply Wash Solution 1

Add **12 ml of Wash Solution 1** to the column and allow it to pass through.

10a. Apply Wash Solution 2

Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution 2.*

Add **12 ml of Wash Solution 2** to the column and allow it to pass through.

11a. Dry Column

Important Reminder: *Make certain the vacuum level is greater than or equal to 500 mbar (refer to App. 2 for unit conversions).*

Following the wash steps, leave the vacuum on for **10 minutes** to dry the column. If **more than 6 columns** are on the vacuum manifold, dry for at least **20 minutes**.

It is important to completely dry the column to prevent ethanol contamination and allow efficient elution in the final preparation. Depending on the strength of the vacuum source, it may be necessary to increase the vacuum time.

Remove any Wash Solution remaining on the inside of the column with a Kimwipes®.

12a. Elute Plasmid DNA

Transfer the binding column to a clean 50 mL collection tube, provided. Add **3 mL of Elution Solution** or molecular biology reagent water to the column. Refer to Elution Options table below to determine which centrifugation speed is appropriate.

For maximum recovery of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at 3000 × *g* for 5 minutes.

For maximum concentration of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at 1000 × *g* for 5 minutes.

Elution Options

Centrifugation Speed	Typical Volume Recovered	Relative Yield	Relative Concentration
3000 X *g*	2.5 mL	100%	100%
1000 X *g*	1.2 mL	80%	175%

The plasmid DNA is present in the eluate and is ready for immediate use, concentration by precipitation, short-term storage at 2–8 °C, or long-term storage at –20 °C.

Spin Format

7b. Prepare Binding Column

Place a GenElute HP Maxiprep Binding Column into a 50 mL collection tube, provided. Add **12 mL of the Column Preparation Solution** to the column and spin in a swinging bucket rotor at **3000 X *g* for 2 minutes**. Discard the eluate.

8b. Filter Lysate and Bind DNA to Column

Hold the filter syringe barrel over the Binding Column and gently apply pressure to the plunger to **expel half** of the cleared lysate into the column. Pull back slightly on the plunger to stop the flow of the remaining lysate.

Be careful not to overfill. Spin in a swinging bucket rotor at **3000 X *g* for 2 minutes**. Discard the eluate. **Add the rest** of the cleared lysate to the column and **repeat the spin**. Discard the eluate.

Some of the lysate may remain in the flocculent material. It is not necessary to force this residual lysate through the filter syringe.

9b. Apply Wash Solution 1

Add **12 ml of Wash Solution 1** to the column and spin in a swinging bucket rotor at **3000 X *g* for 2 minutes**. Discard the eluate.

10b. Apply Wash Solution 2

Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution 2.*

Add **12 ml of Wash Solution 2** to the column and spin in a swinging bucket rotor at **3000 X *g* for 5 minutes**.

11b. Elute Plasmid DNA

Transfer the binding column to a clean 50 mL collection tube, provided. Add **3 mL** of Elution Solution or molecular biology reagent water to the column. Refer to Elution Options table below to determine which centrifugation speed is appropriate.

For maximum recovery of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at **3000 X *g* for 5 minutes**.

For maximum concentration of plasmid: Centrifuge the column/collection tube unit in a swinging bucket rotor at **1000 X *g* for 5 minutes**.

Elution Options

Centrifugation Speed	Typical Volume Recovered	Relative Yield	Relative Concentration
3000 × *g*	2.5 mL	100%	100%
1000 × *g*	1.2 mL	80%	175%

The plasmid DNA is present in the eluate and is ready for immediate use, concentration by precipitation, short-term storage at 2–8 °C, or long-term storage at –20 °C.

DNA Concentration

Important Reminder: *Alcohol precipitation is only necessary if a more concentrated plasmid preparation is desired.*

Transfer the eluate to a clean centrifuge tube. Please note that the provided Collection Tubes should not be centrifuged above 5000 × *g*.

Add **0.1 volumes** of 3.0 M Sodium Acetate Buffer Solution, pH 5.2, and **0.7 volumes** of isopropanol to the recovered plasmid. Mix well by inversion and centrifuge at ≥15,000 × *g* at 4 °C for 30 minutes. Decant the supernatant, being careful not to disturb the pellet.

Rinse the DNA pellet with **1.5 mL** of 70% ethanol and centrifuge as before for 10 minutes. Carefully decant the supernatant and air-dry the pellet until the residual ethanol has evaporated. Resuspend the DNA pellet in the desired volume of Elution Solution or molecular biology reagent water.

DNA Quantitation

Recovery and purity of the plasmid DNA may be determined by spectrophotometric analysis. The ratio of absorbance at $(A_{260}–A_{320})/(A_{280}–A_{320})$ should be 1.8 to 2.0. The A_{320} reading corrects for any background absorbance including that caused by silica fines in the final product. These fines are common in silica-based systems and will have no effect on most downstream applications. The size and quality of the DNA may be determined by agarose gel electrophoresis or pulse field gel electrophoresis.

References

1. Birnboim, H. C.; Doly, J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **1979**, *7*, 1513–1522.
2. Vogelstein, B.; Gillespie, D. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 615-619.

Troubleshooting Guide

Poor or no plasmid DNA recovery.	**Cause** — The plasmid replication is poor. **Solution** — Confirm that the cells were grown in the appropriate medium with a selective antibiotic under optimized conditions.
	Cause — The antibiotic activity is insufficient. **Solution** — Use fresh antibiotic for growth of overnight cultures. Most antibiotics are light sensitive and degrade during long term storage at 2–8 °C.
	Cause — The culture is too old. **Solution** — Streak a fresh plate from a freezer stock. Pick a single colony and prepare a new culture.
	Cause — The overnight culture density is too low. **Solution** — Confirm that the cells were grown in optimal conditions. It may be necessary to increase the starting volume of culture. An optimal cell mass of 750 is recommended where cell mass equals $A_{600} \times$ mL of culture. See the note in Preparation Instructions 2.
	Cause — The overnight culture density is too high. **Solution** — In some cases depending upon the strain, the plasmid, and the culture medium used, cultures can reach very high densities. Reducing the starting volume of culture may be necessary. An optimal cell mass of 750 is recommended where cell mass equals $A_{600} \times$ mL of culture. See the note in Preparation Instructions 2.
	Cause — The binding columns were spun in a fixed angle rotor or with insufficient *g*-force. **Solution** — Spin Format: binding columns must be spun in a swinging bucket rotor at 3000 × *g* for Steps 7b–11b for liquids to pass through efficiently. See note at beginning of the procedure.
	Cause — Wash Solution 2 is too concentrated. **Solution** — Confirm that Wash Solution 2 was diluted with the specified volume of ethanol. Keep the bottle tightly capped between uses to prevent evaporation.
Lysate is not clear after syringe filtration; binding column becomes clogged.	**Cause** — The overnight culture density is too high. **Solution** — In some cases depending upon the strain, the plasmid, and the culture medium used, cultures can reach very high densities. Reducing the starting volume of culture may be necessary. An optimal cell mass of 750 is recommended where cell mass equals $A_{600} \times$ mL of culture. See the note in Preparation Instructions 2.
	Cause — Solutions were added in the wrong order. **Solution** — Make sure that the Neutralization Solution was added before the Binding Solution.

Elution volume recovery is greater than 3 mL.	**Cause** — Binding column is not being sufficiently dried following the wash steps. **Solution** — Vacuum format: allow the columns to dry for the recommended time. Depending on the vacuum pressure of the house system, longer drying times may be necessary. The drying time for Step 11a was based on a vacuum pressure of vacuum pressure of 743 mbar.
Absorbance readings do not accurately reflect the quantity of the plasmid; $(A_{260}–A_{320})/(A_{280}–A_{320})$ ratio is too high or too low.	**Cause** — Wash Solution 2 is diluted with ethanol containing impurities. **Solution** — Check the absorbance of ethanol between 250 and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing. Impurities may show up in the eluate and may contribute to the absorbance in the final product.
	Cause — The plasmid DNA is contaminated with RNA; RNase A treatment is insufficient. **Solution** — Confirm that the RNase A Solution was added to the Resuspension Solution prior to first use. The RNase A Solution may degrade at high temperatures (>65 °C) or prolonged storage (longer than 6 months at room temperature).
	Cause — The plasmid DNA is contaminated with chromosomal DNA. **Solution** — Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during or after the lysis reaction.
	Cause — The background reading is high due to silica fines. **Solution** — Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Additional bands were seen on the analytical gel.	**Cause** — Some of the supercoiled plasmid DNA has become nicked and/or permanently denatured. **Solution** — Plasmid DNA that has been nicked (covalently opened) will run slower than supercoiled DNA during electrophoresis. A small amount of this species of DNA is common and is suitable for downstream applications. Permanently denatured DNA will migrate ahead of the supercoiled DNA and may not be suitable for downstream applications. Do not allow the lysis reaction to proceed longer than 5 minutes.
Poor performance seen in downstream enzymatic applications.	**Cause** — DNA purification is incomplete. **Solution** — Salts in one or more of the solutions may have precipitated. Heat the solution at 65 °C until dissolved. Cool to room temperature prior to use.

Poor performance seen in downstream enzymatic applications.	**Cause** — The plasmid DNA is permanently denatured; alkaline lysis is prolonged. **Solution** — Do not allow the lysis reaction to proceed longer than 5 minutes.
	Cause — The DNA concentration is too low. **Solution** — Reduce the centrifugation speed during elution; see note in Steps 12a or 11b. Alternatively, precipitate the DNA and resuspend in a smaller volume; see "DNA Concentration" section after Procedure.
	Cause — Ethanol is present in the final elution. **Solution** — Vacuum Format: Increase the drying time of the column after washing (Step 11a). Use a wipe to remove any residual Wash Solution 2 that remains on the side of the column after washing (Step 10a). Spin Format: Increase the centrifuge time after Step 10b to remove any residual Wash Solution 2.
	Cause — High salt concentration in final elution **Solution** — Wash Solution 2 was not added to the binding column following the addition of Wash Solution 1.

Related Products	Catalog No.	Related Products	Catalog No.
Kimwipes® Disposable Wipers	Z18,895-6	Gel Loading Solution	G 2526
LB Broth, Sterile Liquid Media	L 2542	DirectLoad™ Wide Range DNA Marker	D 7058
Water, Molecular Biology Reagent	W 4502	Ethidium bromide, aqueous, 10 mg/ml	E 1510
Endotoxin-Free Water	W3500	TAE Buffer (10X Concentrate)	T 9650
3M Sodium Acetate Buffer Solution, pH 5.2	S 7899	TBE Buffer (10X Concentrate)	T 4415
Isopropanol	I 9030, I 0398 or I 9516	Escort II Transfection Reagent	L 6037
Precast Agarose Gels, 1.0%, 8 well	P 5472	Escort V Kit-Enhanced	E 1029

Appendix 1: Centrifuge Speed Conversion Table

Note: All centrifugation speeds are given in units of g. Please refer to Table 1 for information on converting g-force to rpm. *If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time proportionally. Spin until all liquid passes through the column. A swinging bucket rotor is necessary for Step 12a using the vacuum format and Steps 7b-11b using the spin format.*

Table 1. Conversion of Centrifugal Force (in units of g) to rpm for Common Rotors

Centrifuge	Rotor	Type*	Radius (cm)	rpm at 3,000 x *g*	rpm at 5,000 x *g*
Beckman					
Allegra 6	GH-3.8	SB	20.4	3,631	4,688
Allegra 21(R)	S4180	SB	16.1	4,081	5,268
Allegra 64	F0485	FA	9.0	N/A**	N/A
	F0685	FA	9.7	N/A	N/A
TJ-25	TS-5.1-500	SB	19.0	3,756	4,849
	TA-10-250	FA	13.7	N/A	N/A
Rotors for older Beckman centrifuges	JA-10	FA	15.8	N/A	N/A
	JA-14	FA	13.7	N/A	N/A
	JA-20	FA	10.8	N/A	N/A
	JS-13	FA	14.0	N/A	N/A
IEC	215	SB	13.0	4,537	5,857
MP4(R)	224	SB	35.9	2,733	3,528
PR-7000M	966	SB	24.5	3,310	4,274
B22M	877	FA	12.6	N/A	N/A
Sorvall	HB-4	SB	14.7	4,277	5,522
	HB-6	SB	14.6	4,284	5,531
	HS-4	SB	17.2	3,948	5,097
	SH-80	SB	10.1	5,142	6,639
	GSA	FA	14.5	N/A	N/A
	SA-300	FA	9.7	N/A	N/A
	SA-600	FA	12.9	N/A	N/A
	SE-12	FA	9.3	N/A	N/A
	SL-50T	FA	10.7	N/A	N/A
	SS-34	FA	10.7	N/A	N/A

*SB = swinging bucket; FA = fixed angle
**N/A = not appropriate for application

The correct rpm for unlisted rotors can be calculated using the formula:

$$rpm = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*;
r = radius of the rotor in cm;
rpm = the number of revolutions per minute required to achieve the necessary *g*-force

Appendix 2: Vacuum Pressure Conversion Table

All vacuum pressures are given in millibars (mbar). Please refer to Table 2 for information on converting millibars (mbar) to other pressure units.

Table 2. Conversion of millibars (mbar) to Other Pressure Units

Pressure Unit	500 mb equivalent
Inches of mercury (inch Hg)	14.8
Millimeters of mercury (mm Hg)	375
Pounds per square inch (psi)	7.25
Atmospheres (atm)	0.49
Kilopascals (kPa)	50
Torrs (Torr)	375

Experienced User Protocol

Vacuum Format

❑ **Preparation:**

- Add RNase A to the Resuspension Solution
- Add Ethanol to the Wash Solution 2
- Chill Neutralization Solution

1 Harvest Bacteria

❑ Pellet **150 ml** of an overnight culture at 5,000 x *g*, 10 minutes. Discard supernatant.

2 Resuspend & Lyse Bacteria

❑ Resuspend cells in **12 ml of Resuspension/RNase Solution**. Pipette up and down, or vortex.

❑ Add **12 ml of Lysis Solution** and gently invert 6–8 times to mix. Do not vortex. Allow to clear, 3–5 minutes.

3 Prepare To Clear Lysate

❑ Remove the plunger from a filter syringe and place the barrel in an upright position.

❑ Add 12 ml of Neutralization Solution to the lysed cells and gently invert 6–8 times to mix.

❑ Add 9 ml of Binding Solution and gently invert 1–2 times to mix.

❑ Immediately add the mix to the barrel of the filter syringe and let **sit for 5 minutes**.

4 Prepare Column

❑ Place a binding column onto the vacuum manifold and apply the vacuum.

❑ Add **12 ml of Column Preparation Solution** to the column and allow it to pass through.

5 Bind Plasmid DNA to Column

❑ Hold the filter syringe over the column and gently insert the plunger to expel the cleared lysate. Allow the lysate to pass through the column.

6 Wash to Remove Contaminants

❑ Add **12 ml of Wash Solution 1** to the column and allow it to pass through.

❑ Add **12 ml of Wash Solution 2** to the column and allow it to pass through.

❑ Leave vacuum on for **10 minutes to dry** the column.

7 Elute Purified Plasmid DNA

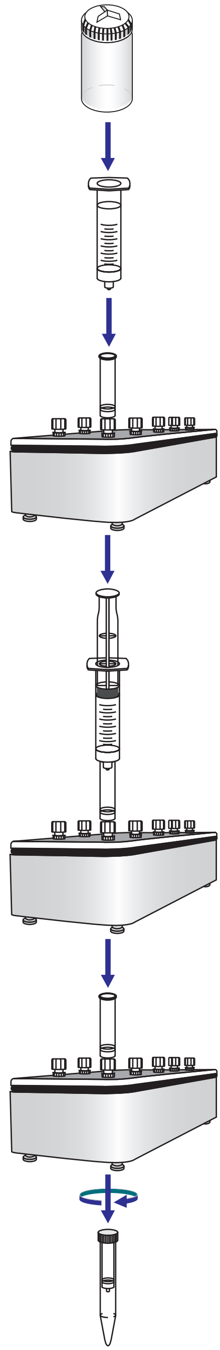
❑ If more than six columns are on the manifold, dry for at least 20 minutes.

❑ Transfer the column to a collection tube provided.

❑ Add **3 ml of Elution Solution** to the Binding Column.

❑ **For maximum yield of Plasmid DNA**: centrifuge the column/collection tube unit in a swinging bucket rotor at 3000 x g for 5 minutes.

❑ **For maximum concentration of Plasmid DNA**: centrifuge the column/collection tube unit in a swinging bucket rotor at 1000 x g for 5 minutes.



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